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K-vitamins or K-quinones

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K-vitamins are important life-molecules, responsible for blood-coagulation, spelt as Koagulation in German, the first syllable of which signifies K class of vitamins. It is curious that most of them are quinones, which have been extensively studied in this laboratory. As such, their spectral studies were undertaken with a view to seek and establish a link between their physical and bio-physical properties. However, they are rare chemicals not easily procurable and but for a generous gift of K_1, K_3, K_4 and K_5 vitamins from Merck, Germany, present studies could not have been possible The infra-red absorption spectrum of vitamin K_1 in the neat liquid phase in 650-4,000 cm^{-1} range and that of K_3, K_4 and K_5 vitamins in nujol mull in 200-4,000 cm^{-1} range, were record on a Perkin-Elmer 621 grating spectrophotometer equipped with NaCl optics. Assuming C_s symmetry for all the molecules under certain conditions, vibrational analysis has been suggested involving fundamental and combination tones. The important fundamentals for K_3 vitamin are 260, 372, 405, 507, 718, 1167, 1377 and 1592 cm^{-1}, the corresponding values of which in other vitamins are near these values. The combination tones of 260, 405, 507, and 1167 cm^{-1} for K_3 vitamins, have been observed with similar ones in the other vitamins

As the infra-red frequencies in all the K-vitamins studied indicate the preponderance of quinonoid structure in all of them, it is suggested that these K-vitamins should rather be called as K-quinones. Further, on the basis of spectral behaviour, it is suggested that K-vitamins are K-quinones, where a naphthoquinone or a similar ring is perturbed by a methyl-group substituent. Hence, these should be extensively studied to seek what actually causes blood-coagulation, a vital problem of life and death.

1. INTRODUCTION

Quinones are compounds of wide occurrence in nature. On account of the well-established biochemical activities like bacteriostatic[1] and antifungicidal action[2], the toxicity to insects, the inhibitory influence on certain enzymes like carboxylase and ureas, the anti-tumor activity which makes some quinones

useful in cancer-chemotherapy[3], their very importannce in Bio-chemistry is becoming constantly more recognized. Recently it was made known that quinones work as a vital link in electron transport[4] and oxidative phosphorylation[5] mechanisms.

One of the quinone derivatives 2-methyl-1, 4-naphthoquinone, happens to be popularly known as K_3-vitamin and exhibits important property of blood-coagulation. It is curious to point out that the origin of he name of K-vitamin first suggested by Dam[6] in 1935, lies in the fact that in German, Coagulation is spelt with K as Koagulation; the first syllable signifying K class of Vitamins. The other K vitamins are K_1, K_2, K_4 and K_5, etc. of which K_1 and K_2 are quinones obtained from plant and animal origin natural products respectively. whereas K_4 and K_5 are synthetic products.

As human system requires K vitamins for certain physiological actions like blood1coagulation, prevention of Jaundice, Thrombosis and certain illnesses affecting pregnant women and newborn children, K_2 vitamin is synthesized within the human body. In case of its deficiency the other vitamins like K_1, K_3, K_4 and K_5 are administered from outside, of which the most important is K_3. The importance of blood-clotting or blood-coagulation can not be over emphasised for life processes, being intimately connected with brain-haemorrhage and heart-failure causing sudden death.

In view of such a vital role played by these K vitamins, it was thought desirable to investigate their spectral properties with a view to establish a link between their physical and bio-physical properties.

As regards the earlier work, it may be mentioned that attempts have been made for studying few vitamins but no comprehensive and comparative study so far has been made. Hans[7] studied synthetic homologues of K_1 and K_2 vitamins with a view to characterize them. It was in our laboratory that K_3-vitamin was spectroscopically studied for the first time, the sensitized electronic spectra of which were obtained as part of a programme by Singh and Singh[8], but no attention was paid to its K-vitamin properties, to which the attention was later drawn by a different reference. This brought their comprehensive study in the focal plane resulting in the present work.

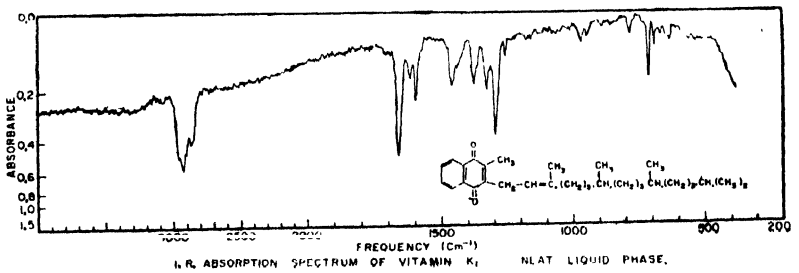
2. Experimental

The work started with a great difficulty so far as procuring the various K vitamins was concerned. It could only be possible through a generous gift of K_1, K_3, K_4 and K_5 vitamins from Messrs E. Merck, D 61, Darmstadt Germany, to whom our thanks are due.

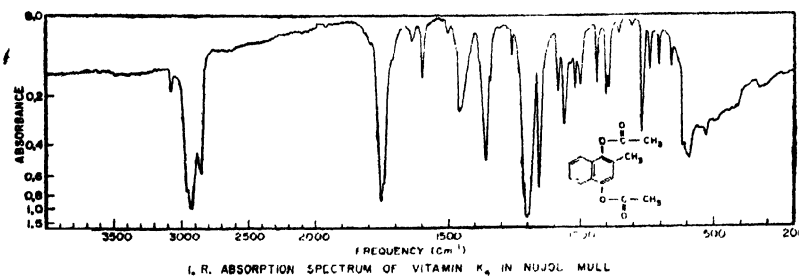
The infra-red absorption spectrum of Vitamin K_1 in the neat liquid phase in 650-4,000 cm^{-1} range and that of K_{1a}, K_4 and K_5 vitamins in nujol mull in 200-4,000 cm^{-1} range have been recorded with a Perkin-Elmer 621 grating spectrophotometer equipped with NaCl optics. In order to achieve better resolution, the spectra were scanned at various optimal speeds. The accuracy involved in the measurement of the absorption peaks lies within $\pm$ 2 cm^{-1} in 200-2,000 cm^{-1} range and that after 2,000 cm^{-1} upto 4000 cm^{-1} falls to around $\pm$ 4 cm^{-1}.

3. Results and Discussion

The various vitamins studied have been structurally depicted alongwith their infra-red traces in figure 1.

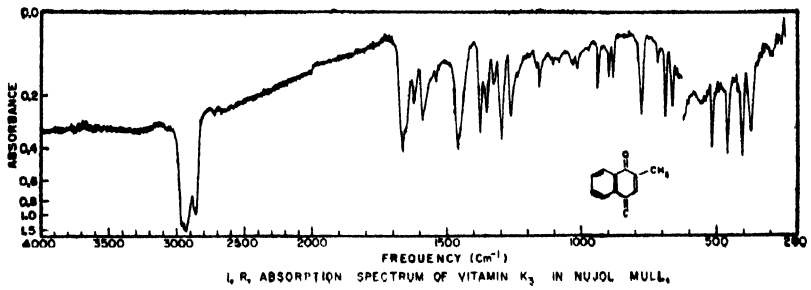


I. R. ABSORPTION SPECTRUM OF VITAMIN K_1 NEAT LIQUID PHASE.

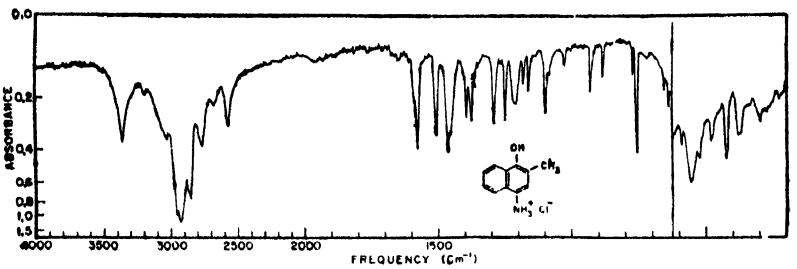


I. R. ABSORPTION SPECTRUM OF VITAMIN K_4 IN NUJOL MULL

The co-ordinates chosen for the molecular rings are those recommended by Mülliken[9], i.e., z-axis being the highest symmetry axis, which in the present case is perpendicular to the molecular plane, assuming the various substituted groups as single entities, as regards the vibrational modes, lying in the molecular plane. On the assumption, all the molecules studied belong to point-group C_s , distribution of the normal modes of which has been given in



I. R. ABSORPTION SPECTRUM OF VITAMIN K_3 IN NUJOL MULL.



I. R. ABSORPTION SPECTRUM OF VITAMIN K_5 IN NUJOL MULL

table 1. As such, all the vibrations are Raman as well as infra-red active. The result has been shown in table 2, where the frequencies of vibrations belonging to the same class have been shown in the same row, for which the same row, for which the mode of vibration and not the approximity in numerical values counted. In the last two columns of table 2, class of vibrations and assignment of frequencies have been given.

Table 1. Distribution of the normal modes of vibrations for K_1, K_2, K_4 and K_5 vitamins, belonging to C_s point-group

Molecule	Point-group	Symmetry Types (species)
Vitamin K_1	C_s	155 a′ + 76 a″
Vitamin K_2	C_s	39 a′ + 18 a″
Vitamin K_4	C_s	63 a′ + 30 a″
Vitamin K_5	C_s	49 a′ + 23 a″

Table 2. Vibrational analysis for K_1, K_3, K_4 and K_5 vitamins

Frequency (cm^{-1})				Species	Assignment
K_1	K_3	K_4	K_5		
663 (w)	663 (mw)	662 (w)	658 (w)	A′	$\nu_1+\nu_2$ (F.R.)
710 (mw)	718 (w)	772 (mw)	772 (w)	a′	C—H bending (o.p.)
782 (w)	775 (mw)	707 (w)	718 (w)	a″	C—H bending (o.p.)
887 (w)	882 (w)	860 (w)	882 (w)	a″	C—H bending (o.p.)
945 (w)	938 (w)	942 (w)	925 (w)	a″	C—H bending (o.p.)
1175 (w)	1167 (w)	1160 (s)	1177 (w)	a′	C—H bending (i.p.)
1255 (mw)	1260 (mw)	1265 (w)	1247 (mw)	a′	C—CH_3 stretching
1292 (s)	1298 (mw)	—	1300 (w)	a′	C—C stretching
1375 (mw)	1377 (mw)	1360 (s)	1375 (mw)	a′	C—C stretching
1458 (mw)	1458 (ms)	1458 (mw)	1452 (ms)	a′	C—C stretching
1593 (ms)	1583 (mw)	1602 (w)	1575 (ms)	a′	C—C stretching
1608 (mw)	1592 (mw)	1633 (w)	1587 (mw)	a′	C—C stretching
1658 (s)	1667 (ms)	1755 (s)	—	a′	C=O stretching
1675 (mw)	1672 (mw)	1773 (mw)	—	A′	$\nu_3+\nu_4$ (F.R.)

ν_1 = 260 cm^{-1}
ν_2 = 405 cm^{-1}
ν_3 = 507 cm^{-1}
ν_4 = 1167 cm^{-1}
w = weak
mw = medium weak
ms = medium strong
s = strong
i.p. = in-plane
o.p. = out-of-plane
F.R. = Fermi-Resonance

The assignments of the fundamental frequencies have been made on the basis of intensity consideration and position of the observed bands.

As a matter of fact vitamin K_1 is nothing but 2-methyl-3-phytyl-1, 4-naphthoquinone and vitamin K_3 is 2-methyl-1-1, 4-naphthoquinone. The analyses of the bands were done on the basis of vibrational assignments of 1, 4-naphthoquinone[8] and as well as on the basis of the work of Hans[7] on synthetic analogues of vitamins K_1 and K_2. Vitamin K_4 and K_5 molecules

resemble substituted 1, 4-naphthohydroquinone with the difference that the former has in place of –OH, $-O-\overset{O}{\overset{\|}{C}}-CH_3$ group at 1 and 4 positions, whereas the latter distinguishes itself from the parent 1, 4-naphthohydroquinone only in that at position 4, it has got instead of -OH group, $-NH^+_3\ Cl^-$ group. Hence in assigning the fundamental modes of vibration to be observed frequencies in the case of vitamins K_1 and K_5 molecules the help has been sought from vibrational work of Hollas[10] on naphthalene, infra-red work of Singh and Singh[11] on β-methyl naphthalene and α-naphtol; also, ester, amino-characteristic[12] group frequencies.

Before discussing the various modes of vibration, it would be worthwhile to point out that bands that appear at 663, 663, 662 and 658 cm^{-1} belonging to vitamins K_1, K_3, K_4 and K_5 respectively may be attributed to Fermi-resonance between fundamentals 260 cm^{-1} (a″ -type) and 405 cm^{-1} (a″ type).

C-H bending modes of vibrations.

Bands involving the in-plane hydrogen bending vibrations absorb at 1300-1000 cm^{-1}, whereas, bands involving the out-of-plane hydrogen bending vibrations absorb from 1000 cm^{-1} to 675 cm^{-1}. In case of molecules of K_1, K_3, K_4 and K_5 vitamins the bands observed at 710, 718, 772 and 772 cm^{-1} (a′ -type); 782, 775, 707 and 718 cm^{-1} (a″ -type); 887, 882, 860 and 882 cm^{-1} (a″ -type); 945, 958, 942 and 925 cm^{-1} (a″ -type), all of weak or medium weak intensity, have been assigned as due to C-H out-of-plane bending. Totally symmetric bands (a′ -type) 1175, 1167, 1160 and 1177 cm^{-1} that appear in the spectra of molecules of K_1, K_3, K_4 and K_5 respectively, have been unambiguously assigned as due to C-H in-plane bending modes of vibration. However, all of them are weak in intensity except one that appears at 1160 cm^{-1} in case of vitamin K_4 molecule and is of strong intensity.

C-CH_3 stretching mode of vibration.

The medium and weak totally symmetric bands at 1255, 1260, 1266 and 1247 cm^{-1} in the case of K_1, K_3, K_4 and K_5 molecules respectively are attributed to C-CH_3 stretching mode of vibration on the basis of vibrational assignments of 1, 4-naphthoquinone[8] and Naphthalene[10].

C—C stretching modes of vibration

Five sets of strong, medium weak and weak but totally symmetric (a′ -type) bands appearing at 1292, 1298 and 1300 cm^{-1}; 1375, 1377, 1360 and 1375

(K_1) (K_3) (K_5)

cm^{-1}; 1458, 1458, 1458 and 1452 cm^{-1}; 1593, 1583, 1602 and 1575 cm^{-1} and 1608, 1592, 1633 and 1587 cm^{-1} belonging to K_1, K_3, K_4 and K_5 vitamins,

are assigned to C-C stretching modes of vibrations. Normally, C-C stretching frequencies are observed in the region 1300-1600 cm $^{-1}$ for 1, 4-naphthoquinone[8] and also in the case of β-methyl naphthalene[11] and α-naphthol[11]. Nevertheless, the appearance of bands at 1602, 1608 and 1633 cm $^{-1}$ may be accounted for

(K_4) (K_1) (K_4)

by C—C stretching mode of vibrations as because the substituents are conjugated[12] $-O-\overset{\overset{O}{\|}}{C}-CH_3$ or phytyl chain (K_1) and appear predominantly as such

(K_4)

there is absorption at these frequencies.

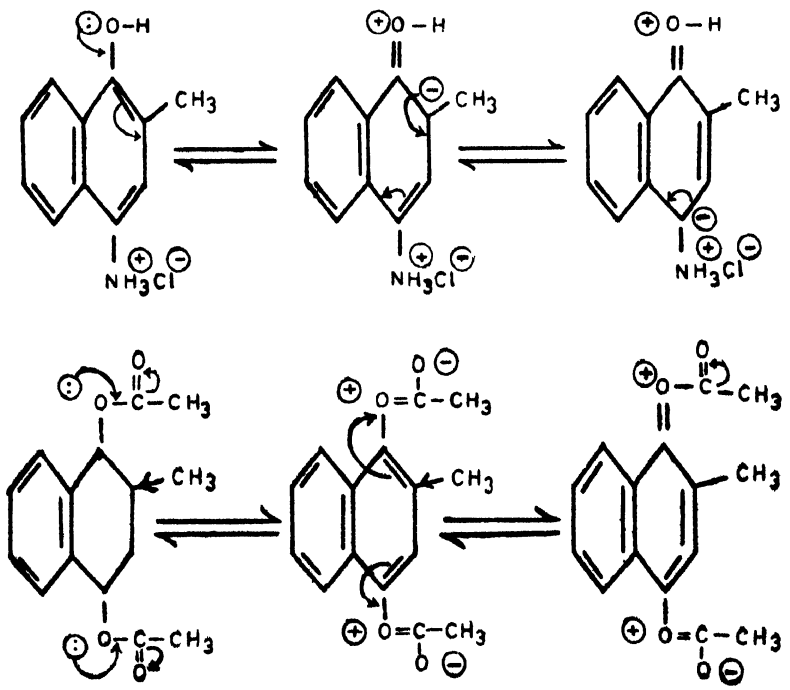
C = O stretching modes of vibrations

The two sets of frequencies 1658, 1667 and 1755 cm $^{-1}$, also 1675, 1672 and 1,773 cm $^{-1}$ of strong and medium weak intensities respectively are observed in the C=O stretching region (1600-1800 cm $^{-1}$) for the molecules of K_1, K_3 and K_4 only. The former set belongs to totally symmetric (a′) class of vibrations, whereas, the latter is of anti-symmetric (a″-type). The cause of splitting of the two carbonyl frequencies has been proposed by Josien *et al*[12] as due to unsymmetrical subsitution of $\diagdown C=O \diagup$ groups. giving rise to two non-equivalent resonating carbonyl systems. Bagli[11] has suggested the cause of splitting as due to intramolecular vibrational effects in quinones and has given reasonable interpretation of the possibility of Fermi-resonance between C=O fundamental and a nearly degenerate combination. Thus the band at 1672 cm $^{-1}$ is interpreted as Fermi-resonance between a′ fundamental and 507 (a′) + 1167 (a′) = 1674 cm $^{-1}$.

However, it may be pointed out that the C=O stretching frequencies 1755 cm $^{-1}$ and 1773 cm $^{-1}$ for vitamin K_1 molecules are characteristics of ester group $O-\overset{\overset{O}{\|}}{C}-CH_3$ linked with aromatic ring but exhibiting aliphatic trend. Moreover, this must be noted that when an electron withdrawing group such as that in case of K_4 molecule is placed on the single bonded oxygen, the carbonyl frequency will be raised[12].

Thus on the basis of common infra-red spectral features following conclusion may be drawn in favour of K-vitamins being renamed as K-quinones. The functional group $\diagdown C=O \diagup$ that is invariably, present in quinone ring causes the π-electrons of carbon atoms to be localized on them rather than making it possible for π-electronic could to get smeared over the whole molecular

frame; the appearance of two C=O sets of frequencies viz. 1658, 1667 and 1755 cm^{-1} belonging to totally symmetric class of vibrations (a′ -type) and 1675, 1672 and 1773 cm^{-1} of antisymmetric (a″ -type) class, of course, degeneracy being removed by perturbation caused due to unsymmetrical substitution in the ring relative to $\diagdown C{=}O \diagup$ positions signifies their quinonic character. It would be appropriate to mention at this juncture that $\diagdown C{=}O \diagup$ characteristic vibrations, determining quinone properties are obtained only in the case of K_1, K_3 and K_4 molecules. The other persistent bands 663, 718, 775, 882, 938, 1167, 1260, 1298, 1377, 1458, 1583 and 1592 cm^{-1} of vitamin K_3 molecules common to remaining K_1, K_4 and K_5 molecules, make them behave as quinones. Further it may be remarked that vitamins K_1 and K_3 molecules which are obviously quinones turn out to be potent and efficacious K vitamins vis-a-vis vitamin K_4 and K_5 vitamins which exhibit but feeble K-activity. The optimal infra-red frequencies, obtained in all the four K-vitamins studied, may go a long way in suggesting for the efficacious synthetic K-product. The spectral standpoint necessitating renaming of K-vitamins as K-quinones is further supported by the very consideration that, though, K_1 and K_3 are as a matter of fact quinones, resonance in structures of vitamins K_4 and K_5 makes them partially behave as quinones, which may be seen from the resonating structures shown in figure 2. As the infra-red



frequencies in all the K-vitamins studied indicate the preponderance of quinonoid structure in all of them, it is suggested that these K-vitamins should rather be called as K-quinones Therefore, it may be concluded that the K-vitamins are K-quinones where a naphthoquinone or a similar ring is perturbed by a methyl group substituent

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